Involvement of β-catenin and unusual behavior of CBP and p300 in glucocorticosteroid signaling in Schwann cells

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In the nervous system, glucocorticosteroid hormones play a major role during development and adult life. Myelin-forming cells are among the targets of glucocorticosteroids, which have been shown to promote myelination both in the central and peripheral nervous system. Glucocorticosteroid-stimulated gene transcription is mediated by the glucocorticosteroid receptor (GR) that recruits coactivators of the p160 family, forming a docking platform for secondary coactivators, such as cAMP-response element binding protein (CREB)-binding protein (CBP) or its close homologue, p300. Here, we investigated the role of CBP and p300 in mouse Schwann cells (MSC80). We show that, although the CBP/p300 binding domain of steroid receptor coactivator-1 is crucial for GR transactivation, neither CBP nor p300 enhanced GR transcriptional activation, as shown by overexpression and small interfering RNA (siRNA) knocking-down experiments. Unexpectedly, overexpression of p300, considered as a coactivator of the GR, resulted in inhibition of GR transcriptional activity. Studies with p300 deletion mutants demonstrated that p300-dependent repression is related to its acetyltransferase activity. Functional and pull-down assays showed that β-catenin may be the coactivator replacing CBP in the GR transcriptional complex. Our results suggest the formation of a GR-coactivator complex within Schwann cells, indicating that glucocorticosteroids may act by means of unusual partners in the nervous system, and we show a repressive effect of p300 on nuclear receptors.

β-catenin | coactivator | glucocorticosteroid receptor | nervous system

Throughout life, glucocorticosteroids (GCs) play a major role in the nervous system. They act by regulating energy metabolism, cell proliferation, and immune responses, and they also promote transcription of genes expressed in neurons and glial cells. GCs enhance myelin formation in both the central and peripheral nervous system (1, 2), as suggested by studies in cultured glial cells (3, 4), and they also stimulate the proliferation of Schwann cells (5). In most cases, GC actions are mediated by their cognate nuclear receptor, the GC receptor (GR). Binding of GCs to GR provokes the interaction with glucocorticosteroid response elements (GREs) in the promoter region of target genes and the recruitment of specific coactivators, such as p160 family members. The GR–p160 complex recruits secondary coactivators, cAMP-response element binding protein (CREB)-binding protein (CBP) or its close homologue, p300, which harbors histone acetyltransferase (HAT) activity. However, the detailed molecular mechanisms underlying GR actions in the nervous system, and in particular in myelinating glial cells, are poorly understood and need to be explored.

Both CBP and p300 are expressed in the nervous system (6), where they play important physiological roles. For example, CBP homozygous mutant mice show complete embryonic lethality and display open neural tube defects (7). Mutations within the CBP gene have been detected in Rubinstein–Taybi patients (8). In Huntington's disease, truncated forms of huntingtin protein in the cell nucleus alter gene transcription by chelating CBP (9, 10). The loss of function of CBP HAT activity could also result in neuronal apoptosis and degeneration (11).

Evidence for a role of β-catenin in steroid receptor signaling has begun to emerge; in fact, it was described to be a coactivator for the androgen (12) and estrogen (13) receptors. β-catenin is a key effector of the canonical Wnt signaling pathway, which plays a fundamental role in brain development. Wnt activation induces β-catenin accumulation and translocation to the nucleus, where it binds to the T cell factor (TCF)/lymphoid enhancer factor family members and activates transcription of target genes.

The aim of the present work was to study the functional interactions between the GR, CBP, and p300 in the immortalized mouse Schwann cell line MSC80. These cells exhibit normal Schwann cell characteristics and retain the capacity to myelinate axons *in vivo* (14). After transplantation into the mouse spinal cord, they show migratory behavior similar to that observed with physiological Schwann cells (15). Moreover, MSC80 cells only express the GR and no other steroid hormone receptor (16), thus facilitating a selective study of the GC signaling pathway. We show that CBP and p300, although both expressed in MSC80 cells, do not act as coactivators of the GR. Unexpectedly, overexpression of p300 resulted in inhibition of GR transcriptional activity, and we demonstrated that its acetyltransferase activity accounts for this repression. Functional experiments showed that β-catenin acts as a coactivator of the GR in MSC80 cells. Moreover, we provide evidence of physical interaction between β-catenin and steroid receptor coactivator-1 (SRC-1), suggesting that β-catenin is a coactivator of the GR in Schwann cells.

Materials and Methods

Cell Culture. The mouse Schwann cell line (MSC80) was maintained in DMEM, supplemented with 10% FCS (Invitrogen), 100 units/ml penicillin, 100 μl/ml streptomycin (Invitrogen), and 0.5 μg/ml fungizone (Invitrogen).

Abbreviations: GR, glucocorticosteroid receptor; CREB, cAMP-response element binding protein; CBP, CREB-binding protein; GC, glucocorticosteroid; GRE, glucocorticosteroid response elements; HAT, histone acetyltransferase; TCF, T cell factor; SRC, steroid receptor coactivator; siRNA, small interfering RNA; CAT, chloramphenicol acetyltransferase; HDAC, histone deacetylases; Dex, dexamethasone.

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Plasmids. Expression vectors of wild-type and mutant SRC-1 have been described by Chauchereau *et al.* (17). CBP and p300 were subcloned in the pSG5 expression vector. E1A and E1A-mut-CBP expression vectors were a generous gift from T. Kouzarides (Gordon Institute, University of Cambridge, Cambridge, U.K.) (18). p300 ΔCRD1 was a gift from N. D. Perkins (University of Dundee, Dundee, U.K.) (19). The p300 ΔE1A, p300 ΔBrD, p300m1HAT, and p300m2HAT were gifts from V. Ogryzko (Centre National de la Recherche Scientifique, Villejuif, France) (20). The β-catenin expression vector and pGEX β-catenin were gifts from M. A. Buendia (Institut Pasteur, Paris) (21, 22). TCF-1 was a gift from S. Rusconi (Fribourg University, Fribourg, Switzerland). The (GRE)2-TATA, ovGRE-tk-chloramphenicol acetyltransferase (CAT), and MMTV-CAT plasmids were described by Massaad *et al.* (23, 24). PGL2-SV40-luciferase vector was purchased from Promega.

Antibodies. The antibodies against CBP (rabbit polyclonal A-22) and p300 (rabbit ploYclonal N-15) were purchased from Santa Cruz Biotechnology. The antibodies against GR (rabbit polyclonal PA1–510A) were purchased from Affinity BioReagents (Golden, CO). The SRC-1 (mouse monoclonal) IgGk were purchased from Upstate Biotechnology (Lake Placid, NY). Fluorescent antibodies were purchased from Molecular Probes: Alexa 488 (mouse), Alexa 555 (rabbit), and Alexa 568 (mouse).

Transient Transfections. MSC80 cells were transiently transfected by using the polyethylenimine reagent (Sigma) as described by Grenier *et al.* (25). One day after transfection, cells were incubated with DMEM containing 10% charcoal-treated FCS and the GC agonist dexamethasone (Dex) (10^{-6} M).

Luciferase assay was used to normalize the transfection efficiency. It was performed as described by Massaad *et al.* (24). The CAT activity was determined by using the two-phase assay described by Massaad *et al.* (26).

***In Vitro* Protein Binding Assays.** The pGEX-β-catenin vector was introduced in the BL21 strain of *Escherichia coli* to synthesize the GST-β-catenin fusion protein as described in the manufacturer's instructions (Amersham Pharmacia Biotech). The assay was performed as described by Chauchereau *et al.* (27). [^{35}S]-radiolabeled proteins (TCF-1 and SRC-1) were synthesized by the transcription of expression vectors and subsequent translation by using the TNT T7 coupled reticulocyte lysate system (Promega) as described by the manufacturer. Protein–protein interactions were performed by incubating 5 μl of the *in vitro*-translated lysate with 10 μg of the GST fusion protein immobilized on glutathione-Sepharose in binding buffer (20 mM Tris/100 mM NaCl/1 mM EDTA/0.1% Nonidet P-40, pH 8.0). Representative gels were stained with Coomassie blue before being subjected to autoradiography to ensure that equal amounts of GST fusion proteins were included in each reaction.

Immunocytochemistry. MSC80 cells were seeded at the density of 2×10^5 cells in 4-cm^2 glass Lab-Tek wells (Nunc). Dex was added 18 h later, and the experiment was performed as described in ref. 25.

Results

Role of CBP and p300 in the GC Signaling Pathway in MSC80 Cells. The initial aim of our study was to explore the potential implication of CBP and p300 in the GR signaling pathway in MSC80 cells. We inhibited the expression of endogenous CBP or p300 by cotransfecting small interfering RNAs (siRNAs) directed against those proteins. The potency of the siRNA was assessed by cotransfecting MSC80 cells with 2 μg of siCBP and GFP expression vector. The transfected cells were identified by their GFP green staining. As shown in Fig. 1*A*, the empty vector (pSuper) did not inhibit endogenous CBP expression, whereas the siRNA against CBP efficiently inhibited the expression of CBP but had no effect on the expression of p300 (data not shown). The same method was used to assess the inhibitory efficacy of the siRNA directed against p300 (Fig. 1*B*). We then transfected MSC80 cells with the siCBP vector and the (GRE)2-TATA-CAT plasmid. This reporter plasmid drives CAT expression under the control of two GREs in tandem and a TATA box.

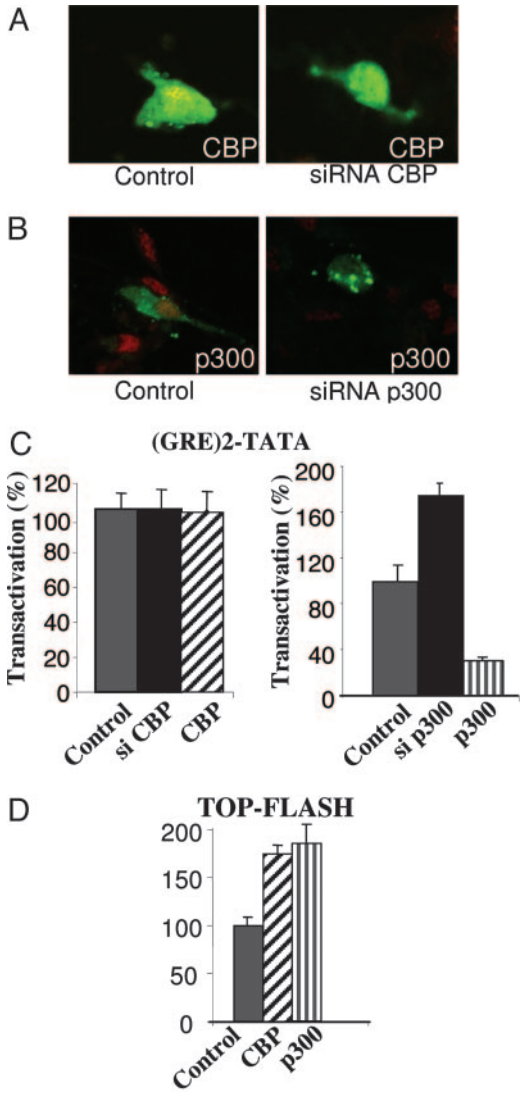

Fig. 1. Role of CBP and p300 in GR signaling: Control of the inhibitory efficiency of siRNAs by immunocytochemistry. (*A* and *B*) MSC80 cells were transiently transfected with GFP expression plasmid (transfected cells appear green) and pSuper (*A Left* and *B Left*), siRNA CBP (*A Right*), or siRNA p300 (*B Right*). Antibodies directed against endogenous CBP or p300 were added overnight. Cells were then incubated for 45 min with secondary antibodies (Cy3, red). The slides were analyzed with a confocal microscope. The experiment was repeated three times; only one is presented here. (*C*) Functionality of CBP and p300. MSC80 cells were transiently transfected with (GRE)2-TATA-CAT plasmid and 1 μg of either CBP or p300 expression vectors or siRNA directed against CBP or p300. Eighteen hours posttransfection, cells were incubated with Dex (10^{-6} M) for 24 h, and then CAT and luciferase activities were assayed. Results represent the mean ± SD of at least four experiments. 100% transactivation represents the normalized CAT activity when empty expression vector is added. (*D*) MSC80 cells were transiently transfected with TOP-FLASH-Luc plasmid and 1 μg of empty, CBP, or p300 expression vectors. CAT and luciferase activities were assayed 36 h later. Results represent the mean ± SD of at least four experiments.

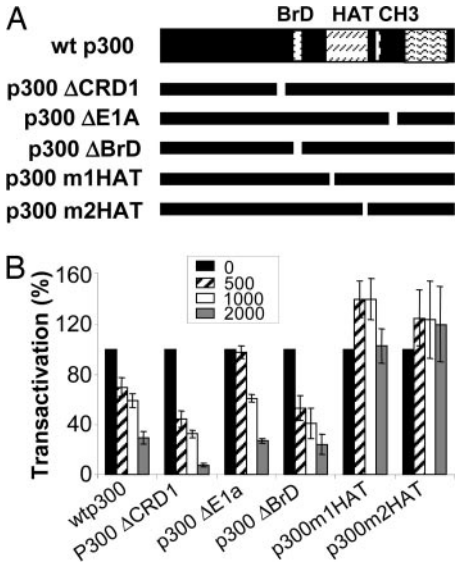

Fig. 2. Identification of p300 repressing domain. (*A*) Schematic representation of wild-type p300 and p300 mutants. (*B*) MSC80 cells were transiently transfected with (GRE)2-TATA-CAT plasmid and increasing amounts (0, 0.5, 1, and 2 μg) of wild-type or mutant p300 expression vectors. Eighteen hours posttransfection, cells were incubated with Dex (10^{-6} M) for 24 h, and then CAT and luciferase activities were assayed. Results represent the mean ± SD of at least four experiments. 100% transactivation represents the normalized CAT activity when empty expression vector is added.

We used this simple construct lacking any transcription factor binding site, except two GREs, to avoid interference between the GR and other transcriptions factors. As shown in Fig. 1*C*, the inhibition of CBP did not affect the transactivation capacity of the GR. Overexpression of CBP expression vector did not significantly enhance the GR-transactivation in MSC80 cells (Fig. 1*C*), whereas it potentiated it by 5-fold in Cos-7 cells (data not shown).

Because p300 is a close homologue of CBP, we investigated whether p300 could replace CBP in the GR complex. Surprisingly, the inhibition of p300 expression by siRNA enhanced ≈2-fold the GR transactivation, whereas overexpression of p300 elicited a transrepression (Fig. 1*C*). These results indicate that p300, a usual coactivator of the GR, may behave as an inhibitor of GR-mediated transcription in Schwann cells.

The absence of effect of CBP and the repressive effect of p300 (80% inhibition) were also observed in the study of two other GC-target promoters, namely the mouse mammary tumor virus-CAT (MMTV-CAT) and a promoter formed by two overlapping GREs (overGRE-Tk-CAT) in MSC80 cells (data not shown). Therefore, we have ruled out the possibility that the repressing effect of p300 depended on the structure of the promoter because two GREs in tandem [(GRE)2-TATA-CAT], overlapping GREs (overGRE-tk-CAT), or half GREs (MMTV-CAT) were all inhibited. To verify the specificity of the unexpected behaviors of CBP and p300 in GR signaling in MSC80, we explored their actions on another transcriptional complex, for which they have also been described as coactivators (28), namely a β-catenin/TCF responding promoter (TOP-FLASH plasmid). Transfection of CBP and p300 in MSC80 cells enhanced the promoter activity by 75% and 60%, respectively (Fig. 1*D*).

Repressing Effect of p300. To understand the mechanism underlying the repressive action of p300, we overexpressed p300 mutants deleted of specific domains (Fig. 2). We tested the potential implication of histone deacetylases (HDAC) in the repressive effect of p300 on the GR. It has been reported that sumoylation of p300, with subsequent recruitment of HDAC6, is responsible for its repressive function toward cyclin-dependent kinase inhibitor p21waf (19). The overexpression of a mutant of p300 lacking its sumoylation domain (p300ΔCRD1) resulted in an inhibition as strong as with wild-type p300. This result shows that the sumoylation domain of p300 is not responsible for GR inhibition. We then overexpressed two mutants of p300 lacking the E1A domain (p300ΔE1A) or the bromodomain (p300 ΔBrD). These two domains are crucial for the recruitment of HDAC1 and histones, respectively. The removal of these two domains did not affect p300 repression of the GR. To exclude any implication of HDACs, we have transfected MSC80 cells with the (GRE)2-TATA-CAT construct and treated the cells with tricostatin A (TSA), a specific inhibitor of HDAC activity. TSA did not result in any significant effect on GR signaling, indicating that the endogenous HDACs were not implicated in GR repression (data not shown). TSA was active in MSC80 cells, because it enhanced the transactivation potency of the Rous sarcoma virus (RSV) promoter (data not shown).

Consequently, we tested the possible implication of p300 HAT activity. We used two mutants of the HAT domain: m1HAT (deletion of nucleotides 1472 through 1522) and m2HAT (deletion of nucleotides 1603 through 1653). MSC80 cells were transiently transfected with the (GRE)2-TATA-CAT plasmid with increasing amounts of either p300m1 HAT or p300m2 HAT deletion plasmids (Fig. 2). Interestingly, these two p300 mutants were devoid of any inhibitory activity on GR signaling, even at high doses of transfected expression vectors. These results demonstrated that the inhibition exerted by p300 on GR signaling was not due to its HDAC activity, but rather to its HAT activity. p300 m1HAT and p300 m2HAT mutants were also tested toward TCF signaling; as expected, they dose-dependently enhanced the transactivation of the TOP-FLASH promoter (data not shown).

Role of CBP in the GR Pathway in MSC80 Cells. As mentioned in Fig. 1, CBP was not implicated in GR transcriptional activity. This lack of coactivating action of CBP was not due to its absence in MSC80 cells, because immunocytochemistry experiments revealed its nuclear expression (Fig. 1). Thus, two hypotheses could be proposed: (*i*) the interaction of the GR with one of the p160 family members may be sufficient to activate transcription; or (*ii*) the p160s could interact with another, yet unknown coactivator to potentiate transactivation. To assess whether SRC-1a, a coactivator of the GR in MSC80 cells (25), could recruit another coactivator instead of CBP, we have cotransfected a deletion mutant of SRC-1a lacking the CBP-interacting domain. Transfection of (GRE)2-TATA-CAT plasmid with increasing amounts of SRC-1ΔCBP/p300 resulted in a dose-dependent inhibition of the reporter plasmid (50% inhibition at 50 ng of transfected expression vector, and 80% inhibition at 0.5 μg of transfected expression vector). This transrepression was due to the replacement of the endogenous SRC-1 by SRC-1ΔCBP/p300, because it dose-dependently inhibited the 2-fold potentiation of transactivation elicited by SRC-1a (data not shown). These results show that the CBP/p300 binding domain of SRC-1 is crucial for the GR transactivation pathway in Schwann cells, where it could dock a coactivator different from CBP or p300.

We addressed the question whether β-catenin could be a coactivator for the GR, because it was previously described as a coactivator for the androgen (12) and estrogen receptors (13). We have cotransfected MSC80 cells with (GRE)2-TATA-CAT or MMTV-CAT constructs and increasing amounts of β-catenin expression vector. In the case of the (GRE)2-TATA promoter, β-catenin dose-dependently potentiated the GR transactivation up to 2.5-fold (Fig. 3*A*). In the case of the MMTV promoter, β-catenin moderately potentiated the GR signaling. As expected, overexpression of

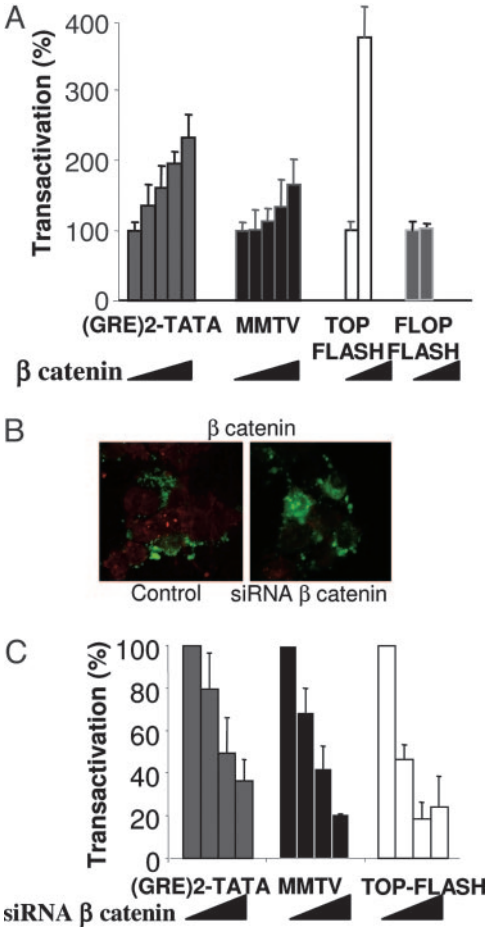

Fig. 3. Role of β-catenin in the GC pathway in MSC80 cells. (*A* and *C*) MSC80 cells were transiently transfected with (GRE)2-TATA-CAT, MMTV-CAT, TOP-FLASH-Luc, or FLOP-FLASH-Luc plasmids and increasing amounts of either β-catenin expression vector (0, 0.5, 1, 2, or 3 μg) (*A*) or siRNA directed against β-catenin (0, 0.5, 1, 2, or 3 μg) (*C*). Cells transfected with (GRE)2-TATA-CAT or MMTV-CAT were incubated with Dex (10^{-6} M) for 24 h. 100% is the activation elicited by Dex, with transfection of the empty expression vector. Results are the mean ± SD of four independent experiments performed in duplicate. (*B*) Control of the inhibitory efficiency of siRNA against β-catenin by using immunocytochemistry. MSC80 cells were transiently transfected with GFP expression plasmid (transfected cells appear green) and either with pSuper or siRNA β-catenin (2 μg). Antibody directed against β-catenin was added overnight. Cells were then incubated during 45 min with secondary antibodies (Cy3, red). The slides were analyzed with a confocal microscope. The experiment was repeated three times, and a typical experiment is presented here.

β-catenin in MSC80 activated the TOP-FLASH promoter but failed to activate the FLOP-FLASH promoter lacking functional TCF binding sites (Fig. 3*A*). We have then inhibited endogenous β-catenin expression by specific siRNA. The efficiency of the designed siRNA was assayed by immunofluorescence (Fig. 3*B*). We have also assayed the potency of β-catenin inhibition by the siRNA vector toward the canonical Wnt signaling. Overexpression of siRNA against β-catenin dose-dependently inhibited TOP-FLASH plasmid activity (80% inhibition at 3 μg of siRNA β-catenin expression vector), indicating that the designed siRNA was efficient in inhibiting β-catenin action (Fig. 3*C*). Knocking-down of β-catenin expression also elicited a dose-dependent repression of GR signaling: 65%-inhibition for the (GRE)2-TATA promoter and 80%-inhibition for the MMTV promoter. Collectively, overexpression of β-catenin as well as its knockdown show that β-catenin is essential for GR signaling in Schwann cells.

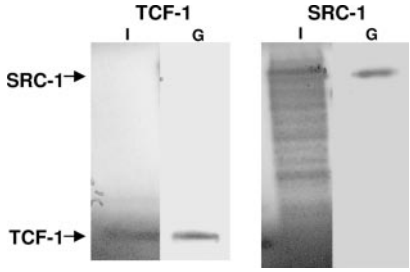

Fig. 4. SRC1 and β-catenin interact *in vitro*. *In vitro*-translated [^{35}S]TCF and SRC1a were incubated with GST control protein or GST-β-catenin fusion protein immobilized on glutathione beads as described in *Materials and Methods*. Bound proteins were analyzed by SDS/PAGE and autoradiography. I, input, total amount of [^{35}S]TCF or SRC1a used for the incubation with the beads; G, GST-β-catenin.

As the deletion of CBP/p300 binding domain of SRC-1 abolished GR transactivation, it was important to check whether β-catenin interacts with SRC-1 docking protein. Therefore, we performed pull-down experiments. We prepared radiolabeled SRC-1a and TCF by using an *in vitro* translation system. GST-β-catenin or GST alone were used to assay the interaction. As shown in Fig. 4, GST-β-catenin was able to interact with TCF and SRC-1a. Control GST did not interact with either protein. The transfections as well as the pull-down assays strongly suggest that β-catenin is a GR coactivator that replaces CBP in MSC80 cells.

Because we have shown that Wnt/TCF and GR pathways recruit β-catenin, we asked whether overexpression of TCF could result in squelching of endogenous β-catenin in MSC80 and consequently impede GR signaling. We therefore cotransfected increasing amounts of TCF-1 expression vector and assayed the (GRE)2-TATA and MMTV promoters. As shown in Fig. 5*A*, TCF-1 overexpression dose-dependently inhibited the activities of these promoters. As expected, TCF-1 enhanced TOP-FLASH promoter, which contains binding sites for TCF. These observations suggest that overexpressed TCF-1 may have sequestered β-catenin, making it unavailable for the

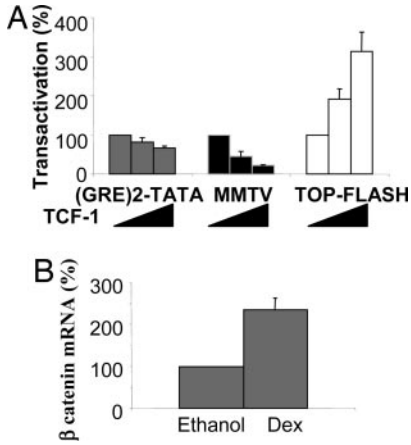

Fig. 5. Crosstalk between β-catenin and GC pathways in MSC80 cells. (*A*) MSC80 cells were transiently transfected with (GRE)2-TATA-CAT, MMTV-CAT, or TOP-FLASH-Luc plasmids and increasing amounts (0, 0.5, 1, and 2 μg) of TCF-1 expression vector. Cells were incubated with Dex (10^{-6} M) for 24 h. 100% is the activation elicited by Dex with transfection of empty expression vector. Results are the mean ± SD of four independent experiments performed in duplicate. (*B*) MSC80 were treated during 24 h with Dex (10^{-6} M) or ethanol. Total RNA was prepared, and Q-PCR was performed to quantify β-catenin transcripts. 26S RNA was used for normalization. Results are the mean ± SD of four independent experiments performed in duplicate. 100% is basal expression of β-catenin transcripts after treatment with ethanol.

GR signaling. We addressed the question of whether the GR is also able to regulate β-catenin levels in MSC80 cells. Real-time PCR experiments, by using total RNA from either ethanol or Dex-treated cells, show that β-catenin mRNA was stimulated 2.5-fold by Dex, suggesting that GR is capable to enhance β-catenin expression for its own use (Fig. 5*B*).

Discussion

In this report, we have evaluated the ability of the GR to interact with the coactivators CBP and p300 in immortalized Schwann cells (MSC80). Interactions with p300 or CBP are known to be essential for the ability of GR to activate its target genes. Our study presents evidence that CBP and p300 can have distinct roles in the regulation of GR transcriptional activity. Several observations ruled out the participation of CBP in GR signaling: (*i*) siRNA directed against CBP did not affect GR signaling; (*ii*) E1a, an adenoviral protein that blocks CBP did not affect Dex-stimulation (data not shown); and (*iii*) overexpression of CBP did not further enhance GR-signaling. In contrast, siRNA-depletion of endogenous p300 enhanced, whereas overexpression of p300 repressed, GR-target promoter activity. These effects are specific to GR, because both CBP and p300 normally activated the TOP-FLASH promoter in MSC80 cells. The mechanism by which p300 inhibits GR transcription does not involve small ubiquitin-like modifier protein (SUMO)-mediated HDAC6 recruitment by p300 as described for p21waf (19), because overexpression of a non-sumoylable p300 mutant inhibited transcription to the same extent as wild-type p300. Moreover, the bromodomain of p300 and the recruitment of HDACs by this latter were not involved, as demonstrated by the deletion of the bromodomain and the treatment of MSC80 cells by HDAC inhibitor tricostatin A. The repressive effect of p300 seems to depend on its HAT activity, because p300 HAT deletion mutants lost the ability to inhibit GR action.

We have previously shown that SRC1a is the p160 family member recruited at the (GRE)2-TATA promoter, but that SRC-1a alone is not sufficient to perform a potent transactivation because the removal of the CBP/p300 binding domain from SRC1 completely abolishes its coactivation ability and transforms it into a dominant-negative form of SRC-1 when overexpressed. We speculate that the SRC-1 may bind a coactivator other than CBP, which could be β-catenin because SRC-1 and β-catenin are able to interact physically. The role of β-catenin as a coactivator of androgen receptor has been well established in prostate cancer cells. Through their interaction, β-catenin augments androgen receptor (AR)-mediated transcription (29–33), and a functional interaction between ER α- and β-catenin has also been recently described in human colon and breast cancer cells (13) and uterus (34).

In our study, we provide evidence supporting the hypothesis that β-catenin may act as a GR-coactivator in MSC80 cells. We show that overexpression of β-catenin potentiated the transcriptional activity of GR. This observation was further confirmed by the decrease of GR transcriptional activity as a result of β-catenin mRNA extinction by specific siRNA. The effect is specific because siRNA against β-catenin has no effect on the promoter in the absence of Dex treatment or on minimal TATA-CAT control plasmid (data not shown).

We also addressed the question of a potential crosstalk between GR and Wnt/TCF signaling pathways in Schwann cells, because both pathways recruit β-catenin. Because Wnt ligands are unavailable, we activated this signaling pathway by overexpressing the transcription factor TCF-1, which is downstream of canonical Wnt signaling. Overexpression of TCF-1 enhanced TOP-FLASH promoter transactivation but elicited a dose-dependent repression of GRE-containing promoters. Our results show that the activation of Wnt signaling, via TCF, impedes GR pathway in MSC80 cells, but not vice versa. This discrepancy may be due to the fact that Dex alters the levels of endogenous β-catenin mRNA in MSC80 cells. In fact, at 10^{-6} M, Dex caused a 2.5-fold increase in β-catenin mRNA, and we also observed a partial transfer of β-catenin into the nucleus after Dex treatment, which favors the increase of the transcriptionally active nuclear pool (data not shown). We speculate that the enhancement of β-catenin levels and its nuclear redistribution in MSC80 cells would overcome its intervention in Wnt/TCF pathway. Dex effects on β-catenin levels were recently studied in several tissues and were shown to be dependent on the cellular type and context. Recent data show that GCs suppresses the canonical Wnt signal in cultured human osteoblasts (35), but others have shown that in rat mammary epithelial tumor cells, Dex up-regulates β-catenin protein and transcript expression, and induced a membrane, rather than a nuclear, localization of β-catenin (36).

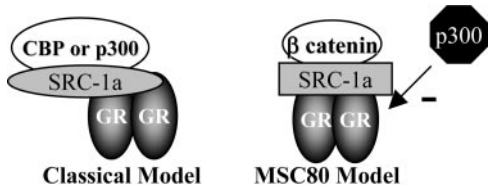

Fig. 6. Schematic model of the GR-coactivator complex. (*A*) Classical model: The GR interacts with the C-terminal nuclear receptor box of SRC-1a, which is a docking platform for CBP or p300. (*B*) MSC80 model: The GR interacts with one or both nuclear receptor boxes of SRC-1a; this interaction may alter SRC-1a conformation and enhance its affinity for β-catenin. p300 acts as a repressor of GR.

Why is CBP excluded from the GR complex in MSC80 cells? It has been described that the GR interacts with SRC-1a exclusively by means of the nuclear receptor (NR) box located in the C-terminal domain of SRC-1a (37, 38). We have shown that, in MSC80 cells, the GR interacts with SRC-1 in an unusual manner, by means of its two NR boxes (25). This atypical interaction may alter the conformation of SRC-1a and consequently may influence the docking of secondary coactivators. Accordingly, interaction between SRC-1a and β-catenin may be promoted. A schematic representation of the MSC80 model is drawn in Fig. 6.

Another interesting result is the repressive effect of p300 on GR signaling because of its HAT activity. It is tempting to speculate that p300 could exert its repressive action at least in part by acetylating β-catenin. Recent data show that β-catenin is one of the substrates of p300, and the acetylation of β-catenin strengthens its binding to TCF4 and decreases its affinity for the androgen receptor (21, 22). Several studies suggest that cooperation between β-catenin and CBP/p300 might depend on the cell and promoter context (10, 28). Other substrates could be acetylated by p300 like PLZF (39)

We provide data strongly supporting the hypothesis of β-catenin acting as a coactivator and p300 as a repressor of GR in glial cells. These effects seem not to be specific for the GR ligand, as they could be observed in the presence of both Dex and the GR pure agonist RU28362 (data not shown). As the recruitment of coregulators by the GR is determinant for the agonistic/antagonistic effects of synthetic drugs, our description of a GR-coactivator complex in Schwann cells could lead to the development of new GC analogs with selective actions upon these cells.

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